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Dear Sir/Madam:

As a follow-up to our previous 8(e) submission dated September 5, 2002, submitted for two polycarbodiimide polymers, I am enclosing one copy of each of the following final reports entitled:

"Skin Sensitization In The Guinea Pig – Magnusson and Kligman Maximisation Method" – September 24, 2002 (CT-720-02)

"Skin Sensitization In The Guinea Pig – Magnusson and Kligman Maximisation Method" – September 24, 2002 (CT-721-02)

These reports **do** contain confidential business information; therefore, a sanitized copy of each report is enclosed for the public record.

Please direct all communications on this subject to me at the address above or call at (973) 357-3375.

Sincerely,

Patricia Ann Vernon
Manager, Regulatory Toxicology Programs

8EHQ-02-15193
8903000000045

SafePharm Laboratories

(CT-720-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 971/180

AUTHOR: R Driscoll

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QUALITY ASSURANCE REPORT

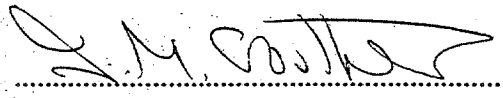
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	28 March 2002	Standard Test Method Compliance Audit
	06 August 2002	Test Material Preparation
	20 August 2002	Animal Preparation
	27 August 2002	Dosing
	08 August 2002	Assessment of Response
§	05 September 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 24 SEP 2002

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA; R Hurst MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

R Driscoll ..... DATE: 24 SEP 2002

R Driscoll BTech (Hons)
Study Director

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(CT-720-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

Method. Twenty test and ten control animals were used for the study. Two phases were involved in the main study; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as:

Intradermal Induction	:	0.1% v/v in arachis oil BP
Topical Induction	:	undiluted as supplied
Topical Challenge	:	undiluted as supplied and 75% v/v in arachis oil BP

Conclusion. Under the conditions of the test, the test material produced a 75% (15/20) sensitisation rate and was classified as a strong sensitiser to guinea pig skin.

The test material was classified as a sensitiser according to EU labelling regulations Commission Directive 93/21/EEC. The symbol "Xi", indication of danger 'irritant' and the risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

(CT-720-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 8). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 18 July 2002 and 24 August 2002.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	(CT-720-02)
Description	:	amber coloured extremely viscous liquid
Batch number	:	DP-26675
Date received	:	24 May 2002
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of this study the test material was used undiluted and freshly prepared in arachis oil BP. The concentrations used are stated in the procedure section.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Thirty-eight male albino Dunkin Hartley guinea pigs were supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK. After an acclimatisation period of at least five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen. At the start of the main study the animals were in the weight range of 280 to 326g, and were eight to twelve weeks old. Nineteen animals were below the weight specified in the Standard Test Method (300g). This deviation was considered not to affect the purpose or integrity of the study.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Certified Guinea Pig Diet (Code 5026) supplied by IPS Product Supplies Limited, Wellingborough, Northants, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, J. Invest. Dermatol. (1969) 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of four guinea pigs, using concentrations of 0.1%, 0.5%, 1% and 5% v/v in arachis oil BP. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the scale shown in Appendix 7. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs (intradermally injected with Freund's Complete Adjuvant nine days earlier) were treated with the undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

3.2.1.3 Selection of Concentration for Topical Challenge

The undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of

erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of thirty guinea pigs was used for the main study, twenty test and ten control. The bodyweight of each animal was recorded at the start and end of the study and are presented in Appendix 6.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line into a 20 mm x 40 mm area. The injections were:

a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1

b) a 0.1% v/v formulation of the test material in arachis oil BP

c) a 0.1% v/v formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie. injection site b) was evaluated according to the scale shown in Appendix 7.

On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the undiluted test material. A filter paper patch (WHATMAN No. 4: approximate size 40 mm x 20 mm), loaded with the undiluted test material was applied to the prepared skin and held in place with a strip of surgical adhesive tape covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale shown in Appendix 7.

Any other reactions were also recorded.

Induction of the Control Animals: The intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

3.2.2.2 Challenge

Shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch (WHATMAN No. 4: approximate size 20 mm x 20 mm), loaded with the undiluted test material was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 75% v/v in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully removed and discarded. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. The position of the treatment sites was identified by using a black indelible marker-pen.

Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

Any other reactions were also recorded.

3.3 Interpretation of Results

Skin reactions noted at the challenge sites of the test group animals will be attributed to skin sensitisation, providing that reactions of equal severity are not seen at the corresponding challenge sites of the control group animals.

If skin reactions are seen at the challenge sites of the control group animals, these will be due to skin irritation, and therefore only skin reactions of greater severity in the test group animals will be attributed to skin sensitisation.

Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.

The sensitisation potential of the test material will be classified as follows:

Percentage of sensitised animals	Classification
0	non-sensitiser
>0 – 8	weak sensitiser
>8 – 28	mild sensitiser
>28 – 64	moderate sensitiser
>64 – 80	strong sensitiser
>80 – 100	extreme sensitiser

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the test and control group animals are presented in Appendix 4.

Discrete or patchy to moderate and confluent erythema was noted at the intradermal induction sites of test group animals.

Discrete or patchy erythema was noted at the intradermal induction sites of control group animals.

5.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the test and control group animals are presented in Appendix 5.

Moderate and confluent erythema and very slight oedema were noted at the topical induction sites of test group animals. A hardened light brown coloured scab was noted at the topical induction sites of two test group animals.

No signs of erythema or oedema were noted at the topical induction sites of control group animals.

Bleeding from the intradermal injection sites was noted in eight test group animals and two control group animals.

5.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of the test and control group animals are given in Table 1.

Undiluted as Supplied

Positive skin responses were noted at the topical challenge sites of ten test group animals. Discrete or patchy to moderate and confluent erythema was noted at the topical challenge sites of ten test group animals at the 24 and 48-hour observations. Very slight oedema was also noted at the topical challenge sites of two test group animals. Desquamation was noted at the topical

challenge sites of seven test group animals at the 48-hour observation. The reaction extended beyond the topical challenge sites of two test group animals at the 24-hour observation and beyond the topical challenge site of one test group animal at the 48-hour observation.

Transient challenge reactions (discrete or patchy erythema) were noted at the topical challenge sites of six test group animals at the 24-hour observation. These reactions were not apparent at the 48-hour observation and were therefore not attributed to contact sensitisation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

75% v/v in Arachis Oil BP

Positive skin responses were noted at the topical challenge sites of fifteen test group animals. Discrete or patchy to moderate and confluent erythema was noted at the topical challenge sites of fifteen test group animals at the 24-hour observation and in fourteen test group animals at the 48-hour observation. Very slight oedema was also noted at the topical challenge sites of three test group animals. Severe desquamation, which prevented evaluation of erythema, was noted at the topical challenge site of one test group animal at the 48-hour observation, this reaction was attributed to contact sensitisation.

Transient challenge reactions (discrete or patchy erythema) were noted at the topical challenge sites of three test group animals at the 24-hour observation. These reactions were not apparent at the 48-hour observation and were therefore not attributed to contact sensitisation.

Desquamation was noted at the topical challenge sites of fourteen test group animals at the 48-hour observation. Reactions extended beyond the challenge sites of two test group animals at the 24-hour observation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

6. CONCLUSION

The test material produced a 75% (15/20) sensitisation rate and was classified as a STRONG SENSITISER to guinea pig skin under the conditions of the test.

The test material produced a sensitisation rate of more than 30% and was classified as a sensitiser according to EU labelling regulations Commission Directive 93/21/EEC. The symbol "Xi", the indication of danger 'irritant' and risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

Table 1 Individual Skin Reactions at Challenge

CHALLENGE CONCENTRATIONS: **Undiluted as Supplied and 75% v/v**

VEHICLE: **Arachis Oil BP**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						75%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	1	0	0	-	0	0	-	1	0	-	1	0	-
	2	1	0	-	0	0	-	1	0	-	1	0	D
	3	0	0	-	0	0	-	1	0	-	0	0	D
	4	0	0	-	0	0	-	1	0	-	1	0	D
	5	1	0	-	1	0	D	2	0	-	2	0	D
	6	2	0	-	1	0	D	2	1	-	2	1	D
	7	2	1	-	2	1	D	2	1	-	2	1	D
	8	1	0	-	1	0	D	1	0	-	1	0	D
	9	2	1	R	2	0	DR	2	1	-	2	1	D
	10	1	0	-	1	0	-	2	0	-	1	0	D
	11	1	0	-	0	0	-	1	0	-	1	0	D
	12	1	0	-	0	0	-	1	0	-	0	0	D
	13	2	0	-	1	0	D	2	0	-	1	0	D
	14	1	0	-	1	0	D	1	0	-	?e	0	D*
	15	0	0	-	0	0	-	0	0	-	0	0	-
	16	1	0	-	1	0	-	1	0	-	1	0	-
	17	1	0	-	0	0	-	0	0	-	0	0	-
	18	1	0	-	0	0	-	2	0	-	1	0	D
	19	1	0	-	0	0	-	1	0	R	0	0	-
	20	1	0	R	1	0	-	1	0	R	1	0	D

Er = Erythema Oe = Oedema - = No other reactions noted R = Reaction extends beyond topical challenge site
D = Desquamation D* = Severe desquamation ?e = Adverse reaction prevents evaluation of erythema

Table 1 (continued) Individual Skin Reactions at Challenge

CHALLENGE CONCENTRATIONS: **Undiluted as Supplied and 75% v/v**

VEHICLE: **Arachis Oil BP**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						75%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er		Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	21	0	0	-	0	0	-	0	0	-	0	0	-
	22	0	0	-	0	0	-	0	0	-	0	0	-
	23	0	0	-	0	0	-	0	0	-	0	0	-
	24	0	0	-	0	0	-	0	0	-	0	0	-
	25	0	0	-	0	0	-	0	0	-	0	0	-
	26	0	0	-	0	0	-	0	0	-	0	0	-
	27	0	0	-	0	0	-	0	0	-	0	0	-
	28	0	0	-	0	0	-	0	0	-	0	0	-
	29	0	0	-	0	0	-	0	0	-	0	0	-
	30	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: Arachis Oil BP

Animal Identification	Concentration of Test Material (% v/v)	Time of Observation	Grade of Erythema at Injection Sites	Evidence of Systemic Toxicity
A	5	24 Hours	N*	None
B	1	24 Hours	3	None
		48 Hours	3*	None
C	0.5	24 Hours	2	None
		48 Hours	3*	None
D	0.1	24 Hours	2	None
		48 Hours	2	None
		72 Hours	2	None
		7 Days	0	None

The concentration of the test material selected for the intradermal induction stage of the main study was 0.1% v/v in arachis oil BP

N = Dermal necrosis * = Animal humanely killed due to severity of reactions

Appendix 2 Topical Sighting Test for Induction Application (48-Hour Exposure) – Individual Skin Reactions

VEHICLE: Arachis Oil BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
E	100	2	0	-	1	0	-	0	0	-
	75	2	0	-	1	0	-	1	0	-
	50	2	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-
F	100	2	0	-	1	0	-	0	0	-
	75	2	0	-	1	0	-	1	0	-
	50	2	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-

The undiluted test material was selected for the main study topical induction

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 3 Topical Sighting Test for Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: Arachis Oil BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
G	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	1	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	2	0	-	0	0	-	0	0	-
H	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	2	0	-	0	0	-	0	0	-

The undiluted test material and a 75% v/v concentration of the test material in arachis oil BP were selected for the main study topical challenge

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 4 Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
TEST	1	1	1	1	1
	2	1	1	1	1
	3	1	1	1	1
	4	1	1	1	2
	5	1	1	1	1
	6	1	1	2	2
	7	1	1	1	2
	8	1	1	1	1
	9	1	1	2	2
	10	1	1	1	1
	11	1	1	2	1
	12	1	1	1	1
	13	1	1	2	2
	14	1	1	1	1
	15	1	1	1	1
	16	1	1	1	1
	17	1	1	1	1
	18	1	1	1	1
	19	1	1	2	2
	20	1	1	1	1

Appendix 4 (continued) Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
CONTROL	21	1	1	1	1
	22	1	1	1	1
	23	1	1	1	1
	24	1	1	1	1
	25	1	1	1	1
	26	1	1	1	1
	27	1	1	1	1
	28	1	1	1	1
	29	1	1	1	1
	30	1	1	1	1

Appendix 5 Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
TEST	1	2	1	-	2	0	-
	2	2	1	Bs	2	0	-
	3	2	1	-	2	0	-
	4	2	1	-	2	0	-
	5	2	1	Bs	2	1	-
	6	2	1	Bs	2	0	-
	7	2	1	-	2	1	-
	8	2	1	-	2	0	-
	9	2	1	-	1	0	-
	10	2	1	-	2	0	-
	11	2	1	Bs	2	0	Sp
	12	2	1	-	2	0	-
	13	2	1	Bs	2	0	-
	14	2	1	Bs	2	0	Sp
	15	2	1	Bs	1	0	-
	16	2	1	-	1	0	-
	17	2	1	-	2	0	-
	18	2	1	-	1	0	-
	19	2	1	Bs	2	0	-
	20	2	1	-	2	0	-

Er = Erythema Oe = Oedema - = No other reactions noted
Bs = Bleeding from intradermal injection sites Sp = Hardened light brown coloured scab

Appendix 5 (continued) Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
CONTROL	21	0	0	-	0	0	-
	22	0	0	Bs	0	0	-
	23	0	0	-	0	0	-
	24	0	0	Bs	0	0	-
	25	0	0	-	0	0	-
	26	0	0	-	0	0	-
	27	0	0	-	0	0	-
	28	0	0	-	0	0	-
	29	0	0	-	0	0	-
	30	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted Bs = Bleeding from intradermal injection sites

Appendix 6 Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 24	
TEST	1	286	434	148
	2	282	469	187
	3	326	571	245
	4	311	459	148
	5	296	477	181
	6	285	483	198
	7	296	455	159
	8	291	490	199
	9	315	480	165
	10	292	447	155
	11	292	485	193
	12	286	412	126
	13	292	425	133
	14	292	461	169
	15	282	458	176
	16	303	462	159
	17	309	516	207
	18	300	485	185
	19	315	457	142
	20	313	442	129

Bodyweight increases of the guinea pigs in the test group between Day 0 and Day 24 were comparable to those noted in the control group animals over the same period.

Appendix 6 (continued) Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 24	
CONTROL	21	301	505	204
	22	298	453	155
	23	293	456	163
	24	311	472	161
	25	280	430	150
	26	295	453	158
	27	281	447	166
	28	280	477	197
	29	296	429	133
	30	314	471	157

(CT-720-02) : SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

Appendix 7 Scales For Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No erythema	0
Barely perceptible erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre extending beyond the area of exposure)	4

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† From: Draize, J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC, p31.

Appendix 8 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Project Number	Date Start	Date End	Number of Animals and Sex*		Positive Control Material	Concentration			Incidence of Sensitisation
						Induction		Challenge	
			Test	Control		Intradermal	Topical		
039/422	12/01/00	05/02/00	10 Female	5 Female	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (10/10)
039/444	29/06/00	22/07/00	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (9/9)
039/446	28/06/00	06/08/00	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	50% (5/10)
039/458	25/01/01	25/02/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	40% (4/10)
039/540	26/09/01	29/10/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	20% (2/10)
039/576	22/05/02	15/06/02	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	80% (8/10)

* All animals supplied by David Hall Ltd, Burton-on-Trent, Staffordshire, UK

Appendix 9 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
SafePharm Laboratories Ltd **Shardlow Business Park** **London Road** **Shardlow** **Derbyshire** **DE72 2GD**	**Analytical Chemistry** **Environmental Fate** **Environmental Toxicity** **Mutagenicity** **Phys/Chem Tests** **Toxicology**

DATE OF INSPECTION
28 February 2000

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

SAFEPHARM LABORATORIES LTD

(CT-720-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 971/180

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 27 SEP 2002

R Driscoll BTech (Hons)
Study Director

SafePharm Laboratories

(CT-721-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 971/177

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QUALITY ASSURANCE REPORT

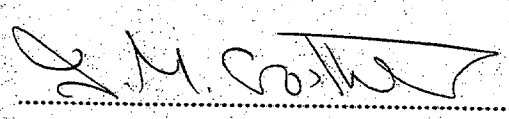
This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	28 March 2002	Standard Test Method Compliance Audit
	06 August 2002	Test Material Preparation
	20 August 2002	Animal Preparation
	27 August 2002	Dosing
	08 August 2002	Assessment of Response
§	05 September 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



..... DATE: 24 SEP 2002

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA; R Hurst MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

R Driscoll ..... DATE: 24 SEP 2002

R Driscoll BTech (Hons)
Study Director

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(CT-721-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

Method. Twenty test and ten control animals were used for the study. Two phases were involved in the main study; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as:

Intradermal Induction	:	0.1% v/v in arachis oil BP
Topical Induction	:	undiluted as supplied
Topical Challenge	:	undiluted as supplied and 75% v/v in arachis oil BP

Conclusion. Under the conditions of the test, the test material produced a 100% (20/20) sensitisation rate and was classified as an extreme sensitiser to guinea pig skin.

The test material was classified as a sensitiser according to EU labelling regulations Commission Directive 93/21/EEC. The symbol "Xi", indication of danger 'irritant' and the risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

(CT-721-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 8). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 18 July 2002 and 24 August 2002.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	(CT-721-02)
Description	:	light amber coloured extremely viscous liquid
Batch number	:	DP-26676
Date received	:	24 May 2002
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of this study the test material was used undiluted and freshly prepared in arachis oil BP. The concentrations used are stated in the procedure section.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Thirty-eight male albino Dunkin Hartley guinea pigs were supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK. After an acclimatisation period of at least five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen. At the start of the main study the animals were in the weight range of 279 to 329g, and were eight to twelve weeks old. Seventeen animals were below the weight specified in the Standard Test Method (300g). This deviation was considered not to affect the purpose or integrity of the study.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Certified Guinea Pig Diet (Code 5026) supplied by IPS Product Supplies Limited, Wellingborough, Northants, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, J. Invest. Dermatol. (1969) 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of four guinea pigs, using concentrations of 0.1%, 0.5%, 1% and 5% v/v in arachis oil BP. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the scale shown in Appendix 7. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs (intradermally injected with Freund's Complete Adjuvant nine days earlier) were treated with the undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

3.2.1.3 Selection of Concentration for Topical Challenge

The undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of

erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of thirty guinea pigs was used for the main study, twenty test and ten control. The bodyweight of each animal was recorded at the start and end of the study and are presented in Appendix 6.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line into a 20 mm x 40 mm area. The injections were:

a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1

b) a 0.1% v/v formulation of the test material in arachis oil BP

c) a 0.1% v/v formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie. injection site b) was evaluated according to the scale shown in Appendix 7.

On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the undiluted test material. A filter paper patch (WHATMAN No. 4: approximate size 40 mm x 20 mm), loaded with the undiluted test material was applied to the prepared skin and held in place with a strip of surgical adhesive tape covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale shown in Appendix 7.

Any other reactions were also recorded.

Induction of the Control Animals: The intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

3.2.2.2 Challenge

Shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch (WHATMAN No. 4: approximate size 20 mm x 20 mm), loaded with the undiluted test material was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 75% v/v in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully removed and discarded. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. The position of the treatment sites was identified by using a black indelible marker-pen.

Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

Any other reactions were also recorded.

3.3 Interpretation of Results

Skin reactions noted at the challenge sites of the test group animals will be attributed to skin sensitisation, providing that reactions of equal severity are not seen at the corresponding challenge sites of the control group animals.

If skin reactions are seen at the challenge sites of the control group animals, these will be due to skin irritation, and therefore only skin reactions of greater severity in the test group animals will be attributed to skin sensitisation.

Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.

The sensitisation potential of the test material will be classified as follows:

Percentage of sensitised animals	Classification
0	non-sensitiser
>0 – 8	weak sensitiser
>8 – 28	mild sensitiser
>28 – 64	moderate sensitiser
>64 – 80	strong sensitiser
>80 – 100	extreme sensitiser

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the test and control group animals are presented in Appendix 4.

Moderate and confluent erythema was noted at the intradermal induction sites of test group animals.

Discrete or patchy to moderate and confluent erythema was noted at the intradermal induction sites of control group animals.

5.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the test and control group animals are presented in Appendix 5.

Moderate and confluent erythema and very slight oedema were noted at the topical induction sites of test group animals.

No signs of erythema or oedema were noted at the topical induction sites of control group animals.

Bleeding from the intradermal injection sites was noted in all test group animals and four control group animals.

5.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of the test and control group animals are given in Table 1.

Undiluted as Supplied

Positive skin responses were noted at the topical challenge sites of fourteen test group animals. Discrete or patchy to moderate and confluent erythema was noted at the topical challenge sites of fourteen test group animals at the 24-hour observation. Discrete or patchy erythema was noted at the topical challenge sites of thirteen test group animals at the 48-hour observation. Severe

desquamation, which prevented evaluation of erythema, was noted at the topical challenge site of one test group animal at the 48-hour observation and the reactions in this animal were attributed to contact sensitisation. Desquamation was noted at the topical challenge sites of five test group animals at the 48-hour observation.

Transient challenge reactions (discrete or patchy erythema) were noted at the topical challenge sites of six test group animals at the 24-hour observation. These reactions were not apparent at the 48-hour observation and were therefore not attributed to contact sensitisation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

75% v/v in Arachis Oil BP

Positive skin responses were noted at the topical challenge sites of all test group animals. Discrete or patchy to moderate and confluent erythema, with or without very slight oedema, was noted at the topical challenge sites of all test group animals at the 24-hour observation and in fourteen test group animals at the 48-hour observation. Severe desquamation, which prevented evaluation of erythema, was noted at the topical challenge sites of six test group animals at the 48-hour observation and the reactions in these animals were attributed to contact sensitisation. Desquamation was noted at the topical challenge sites of nine test group animals at the 48-hour observation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

6. CONCLUSION

The test material produced a 100% (20/20) sensitisation rate and was classified as an EXTREME SENSITISER to guinea pig skin under the conditions of the test.

The test material produced a sensitisation rate of more than 30% and was classified as a sensitiser according to EU labelling regulations Commission Directive 93/21/EEC. The symbol "Xi", the indication of danger 'irritant' and risk phrase R 43 "May Cause Sensitisation by Skin Contact" are therefore required.

Table 1 Individual Skin Reactions at Challenge

CHALLENGE CONCENTRATIONS: **Undiluted as Supplied and 75% v/v**

VEHICLE: **Arachis Oil BP**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						75%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	1	2	0	-	1	0	D	2	0	-	?e	0	D*
	2	1	0	-	0	0	-	2	1	-	1	0	D
	3	1	0	-	1	0	-	2	1	-	1	0	D
	4	1	0	-	0	0	-	2	1	-	1	0	-
	5	2	0	-	1	0	-	2	0	-	1	0	D
	6	1	0	-	1	0	-	1	0	-	1	0	D
	7	1	0	-	1	0	-	2	1	-	2	1	D
	8	1	0	-	1	0	-	1	0	-	1	0	-
	9	2	0	-	1	0	-	2	1	-	1	0	D
	10	2	0	-	1	0	D	2	0	-	1	0	D
	11	2	0	-	?e	0	D*	2	0	-	?e	0	D*
	12	2	0	-	1	0	-	2	1	-	?e	0	D*
	13	1	0	-	0	0	-	1	0	-	1	0	D
	14	2	0	-	1	0	D	2	0	-	?e	0	D*
	15	1	0	-	0	0	-	2	1	-	1	0	-
	16	1	0	-	0	0	-	2	1	-	1	0	-
	17	1	0	-	1	0	D	1	0	-	?e	0	D*
	18	2	0	-	1	0	D	2	1	-	?e	0	D*
	19	1	0	-	0	0	-	2	0	-	1	0	-
	20	1	0	-	1	0	-	2	0	-	1	0	D

Er = Erythema Oe = Oedema - = No other reactions noted
D = Desquamation D* = Severe desquamation ?e = Adverse reaction prevents evaluation of erythema

Table 1 (continued) Individual Skin Reactions at Challenge

CHALLENGE CONCENTRATIONS: Undiluted as Supplied and 75% v/v

VEHICLE: Arachis Oil BP

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		100%						75%					
		24 Hours			48 Hours			24 Hours			48 Hours		
		Er		Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	21	0	0	-	0	0	-	0	0	-	0	0	-
	22	0	0	-	0	0	-	0	0	-	0	0	-
	23	0	0	-	0	0	-	0	0	-	0	0	-
	24	0	0	-	0	0	-	0	0	-	0	0	-
	25	0	0	-	0	0	-	0	0	-	0	0	-
	26	0	0	-	0	0	-	0	0	-	0	0	-
	27	0	0	-	0	0	-	0	0	-	0	0	-
	28	0	0	-	0	0	-	0	0	-	0	0	-
	29	0	0	-	0	0	-	0	0	-	0	0	-
	30	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: **Arachis Oil BP**

Animal Identification	Concentration of Test Material (% v/v)	Time of Observation	Grade of Erythema at Injection Sites	Evidence of Systemic Toxicity
A	5	24 Hours	N*	None
B	1	24 Hours	3	None
		48 Hours	N+*	None
C	0.5	24 Hours	2	None
		48 Hours	3*	None
D	0.1	24 Hours	2	None
		48 Hours	2	None
		72 Hours	2	None
		7 Days	0	None

The concentration of the test material selected for the intradermal induction stage of the main study was 0.1% v/v in arachis oil BP

N = Dermal necrosis N+ = Dark green coloured dermal necrosis * = Animal humanely killed due to severity of reactions

Appendix 2 Topical Sighting Test for Induction Application (48-Hour Exposure) – Individual Skin Reactions

VEHICLE: Arachis Oil BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
E	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-
F	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	1	0	-	0	0	-	0	0	-

The undiluted test material was selected for the main study topical induction

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 3 Topical Sighting Test for Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: Arachis Oil BP

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
G	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	2	0	-	0	0	-	0	0	-
H	100	2	0	-	0	0	-	0	0	-
	75	2	0	-	0	0	-	0	0	-
	50	2	0	-	0	0	-	0	0	-
	25	2	0	-	0	0	-	0	0	-

The undiluted test material and a 75% v/v concentration of the test material in arachis oil BP were selected for the main study topical challenge

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 4 Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
TEST	1	2	2	2	2
	2	2	2	2	2
	3	2	2	2	2
	4	2	2	2	2
	5	2	2	2	2
	6	2	2	2	2
	7	2	2	2	2
	8	2	2	2	2
	9	2	2	2	2
	10	2	2	2	2
	11	2	2	2	2
	12	2	2	2	2
	13	2	2	2	2
	14	2	2	2	2
	15	2	2	2	2
	16	2	2	2	2
	17	2	2	2	2
	18	2	2	2	2
	19	2	2	2	2
	20	2	2	2	2

Appendix 4 (continued) Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
CONTROL	21	1	1	1	1
	22	1	1	1	1
	23	1	1	1	1
	24	1	1	1	1
	25	1	1	1	1
	26	2	2	2	2
	27	2	2	2	2
	28	1	2	1	2
	29	1	1	1	1
	30	1	1	1	1

Appendix 5 Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
TEST	1	2	1	Bs	2	1	-
	2	2	1	Bs	2	1	-
	3	2	1	Bs	2	1	-
	4	2	1	Bs	2	1	-
	5	2	1	Bs	2	1	-
	6	2	1	Bs	2	1	-
	7	2	1	Bs	2	1	-
	8	2	1	Bs	2	1	-
	9	2	1	Bs	1	0	-
	10	2	1	Bs	2	0	-
	11	2	1	Bs	2	1	-
	12	2	1	Bs	2	0	-
	13	2	1	Bs	2	0	-
	14	2	1	Bs	2	0	-
	15	2	1	Bs	1	1	-
	16	2	1	Bs	1	1	-
	17	2	1	Bs	2	1	-
	18	2	1	Bs	1	1	-
	19	2	1	Bs	2	0	-
	20	2	1	Bs	2	1	-

Er = Erythema Oe = Oedema - = No other reactions noted Bs = Bleeding from intradermal injection sites

Appendix 5 (continued) Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
CONTROL	21	0	0	Bs	0	0	-
	22	0	0	-	0	0	-
	23	0	0	Bs	0	0	-
	24	0	0	-	0	0	-
	25	0	0	-	0	0	-
	26	0	0	-	0	0	-
	27	0	0	Bs	0	0	-
	28	0	0	-	0	0	-
	29	0	0	-	0	0	-
	30	0	0	Bs	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted Bs = Bleeding from intradermal injection sites

Appendix 6 Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 24	
TEST	1	286	444	158
	2	300	442	142
	3	298	394	96
	4	296	458	162
	5	292	465	173
	6	295	446	151
	7	286	457	171
	8	295	450	155
	9	294	445	151
	10	280	424	144
	11	284	401	117
	12	298	432	134
	13	288	446	158
	14	279	419	140
	15	293	465	172
	16	305	501	196
	17	302	443	141
	18	329	519	190
	19	296	470	174
	20	305	442	137

Bodyweight increases of the guinea pigs in the test group between Day 0 and Day 24 were comparable to those noted in the control group animals over the same period.

Appendix 6 (continued) Individual Bodyweights and Bodyweight Gains

Group	Animal Number	Bodyweight (g)		Bodyweight (g) Increase
		Day 0	Day 24	
CONTROL	21	318	462	144
	22	311	482	171
	23	313	461	148
	24	317	502	185
	25	290	435	145
	26	290	405	115
	27	300	438	138
	28	315	463	148
	29	323	501	178
	30	324	462	138

(CT-721-02) : SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

Appendix 7 Scales For Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No erythema	0
Barely perceptible erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre extending beyond the area of exposure)	4

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† From: Draize, J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC, p31.

Appendix 8 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Project Number	Date Start	Date End	Number of Animals and Sex*		Positive Control Material	Concentration			Incidence of Sensitisation
			Test	Control		Induction		Challenge	
						Intradermal	Topical		
039/422	12/01/00	05/02/00	10 Female	5 Female	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (10/10)
039/444	29/06/00	22/07/00	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (9/9)
039/446	28/06/00	06/08/00	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	50% (5/10)
039/458	25/01/01	25/02/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	40% (4/10)
039/540	26/09/01	29/10/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	20% (2/10)
039/576	22/05/02	15/06/02	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	80% (8/10)

* All animals supplied by David Hall Ltd, Burton-on-Trent, Staffordshire, UK

Appendix 9 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Tests
Toxicology

DATE OF INSPECTION
28 February 2000

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

SAFEPHARM LABORATORIES LTD

(CT-721-02):

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 971/177

I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

R Driscoll ..... DATE: 27 SEP 2002

R Driscoll BTech (Hons)
Study Director